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Reference intervals of two-dimensional speckle tracking—derived endocardial global longitudinal strain analysis in 132 healthy cats

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Abstract *Introduction:* The assessment of left ventricular myocardial deformation and function by two-dimensional speckle tracking—derived strain analysis is an established method in human cardiology. It also progressively gains recognition in veterinary cardiology in both dogs and cats.
Objectives: The objectives of this study were to create reference intervals for two-dimensional speckle tracking echocardiography (STE)—derived endocardial global longitudinal strain (GLS) in a population of healthy adult cats of different breeds. Influences of heart rate, body weight, and age were investigated.
Animals: A total of 132 healthy, adult cats were included in this study.
Materials and Methods: Left apical two-, three-, and four-chamber views were obtained prospectively for GLS measurements using two-dimensional speckle tracking performed with cardiac performance analysis. Potential influence of body weight, heart rate, and age was analyzed, and the interobserver and intra-observer variability of the measurements was determined.
Results: Endocardial GLS values were not significantly influenced by body weight (P=0.102), heart rate (P=0.144), or age (P=0.075). A reference interval for GLS of −21.18% to −37.50% (±4.12) was determined. The interobserver and intra-observer variability showed excellent agreement.
Discussion and Conclusions: Two-dimensional STE is a feasible technique for the evaluation of cardiac myocardial deformation and systolic function in cats. Showing an excellent interobserver and intra-observer agreement, two-dimensional STE is a promising method for clinical analysis of cardiac deformation in cats.

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Abbreviations

2D	two-dimensional
CPA	cardiac performance analysis
GLS	global longitudinal strain
HCM	hypertrophic cardiomyopathy
RI	reference interval
STE	speckle tracking echocardiography

Introduction

Cardiomyopathies of different phenotypes are the most common cardiac diseases in feline patients [1—3]. Feline cardiomyopathies include hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy, dilated cardiomyopathy, cardiomyopathy of non-specific phenotype, as well as arrhythmogenic right ventricular cardiomyopathy [2,4]. A complex approach is essential for the correct classification. Current reference intervals for cardiac dimensions, pressure conditions, flow velocities, and guidelines for the interpretation of two-dimensional images [5—8] allow screening and classification of feline cardiomyopathies as well as their staging [9—13].

Hypertrophic cardiomyopathy is the most common type of feline cardiomyopathies [2,9,14]. It can present as a generalized, focal, or papillary muscle—associated type [12]. For classification and diagnosis of HCM, other hypertrophic phenotypes, such as transient myocardial thickening, endocrinopathy-induced left ventricular hypertrophy, or hypertensive cardiomyopathy, need to be excluded. Hypertrophic cardiomyopathy might lead to the occurrence of regional ischemia with progression of the disease. With further progression, a transition into end-stage cardiomyopathy presenting with systolic dysfunction and myocardial fibrosis [15] can occur as similarly described in human literature [16]. Recently, feline regional left ventricular thinning was described [17].

Echocardiographic methods such as tissue Doppler imaging or strain measurement are used to detect early stages of diastolic and systolic dysfunction [10,13,18—23]. The first studies that evaluated myocardial deformation were based on color tissue Doppler imaging—derived strain measurements and reported reduced systolic function in feline HCM patients compared to healthy controls [13]. More recently, strain measurements using two-dimensional speckle tracking echocardiography (STE) have been introduced as a more robust method [24]. Speckles are generated by myocardial and ultrasound beam interactions during standard echocardiography. Frame-by-frame changes of their position during the cardiac cycle allow position tracking by specialized software. Speckle tracking—derived strain is used to investigate global or regional myocardial changes in humans [25]. Pattern recognition using bull's eye plots displaying the myocardial strain graphically in 16—18 segments is used for determination of the underlying disease inducing human cardiomyopathies such as amyloidosis or Takotsubo cardiomyopathy [26,27]. Moreover, it is a useful tool in the assessment of HCM in humans [28].

There are also studies investigating the application of two-dimensional STE in healthy feline patients [29—31] and the evaluation of feline HCM by STE [18—20,23,32,33]. Current studies investigating longitudinal strain in cats only used a left apical four-chamber view. In contrast, recent recommendations in human literature prefer an approach measuring longitudinal strain in three different echocardiographic views to get a true global assessment of longitudinal myocardial deformation [34—37].

Therefore, the goal of this study was to establish reference intervals for endocardial global longitudinal strain (GLS) using, as in humans, three different echocardiographic views (two-chamber view, three-chamber view, and four-chamber view). The influence of heart rate, age, and weight on GLS was additionally investigated. The second aim was to evaluate potential differences regarding strain in three different left apical imaging planes.

Materials and methods

The present study was designed as a prospective study. The study design was reviewed and approved by the Ethics Committee of the Department of Veterinary Medicine, Ludwig Maximilians University of Munich (permission number: 214-22-04-2020).

Animals

Healthy client-owned cats presented for routine cardiology checkup or for an echocardiographic breeding examination have been investigated at the Small Animal Clinic of the Ludwig Maximilians University of Munich from 2018 to 2021.

Included cats were classified as being healthy based on medical records, complete clinical examination, as well as a complete echocardiographic examination with simultaneous electrocardiogram recording in right and left lateral recumbency without sedation.

Cats were excluded when diastolic wall thickness, left ventricular systolic, or diastolic chamber diameters were outside established reference intervals [5] or when a diastolic dysfunction based on mitral inflow profiles, tissue velocity imaging measurements, and isovolumetric relaxation time was detected [10]. Furthermore, detection of systolic anterior motion with or without additional left ventricular outflow tract obstruction observed on a two-dimensional image or color Doppler led to exclusion of the animal from the study. Trivial valvular regurgitations detected by color Doppler or dynamic right ventricular outflow tract obstruction were allowed. Any ongoing medication influencing the cardiovascular system led to an exclusion of the study. Cats have been excluded if they showed any cardiac rhythm other than sinus rhythm. All cats had a physiologic body condition score between four and six out of nine.

Echocardiographic examination

All transthoracic echocardiographic studies have been performed by a board-certified cardiologist or cardiology residents under direct supervision.

An ultrasound unit[a] with a 12-MHz transducer was used in all studies. A simultaneous electrocardiogram was recorded, and heart rate was monitored by this means.

Routine echocardiographic measurements [9,38] comprising two-dimensional interventricular septal wall thickness, left ventricular inner diameter, and left ventricular posterior wall thickness both in systole and diastole were performed in every patient using a right parasternal long-axis view. Diameters of the aortic root and left atrium were measured in a right parasternal short-axis view at the level of the heart base, and left atrium-to-aortic ratio was calculated. Standard spectral, color flow Doppler, and tissue velocity imaging were performed as described earlier [10,38].

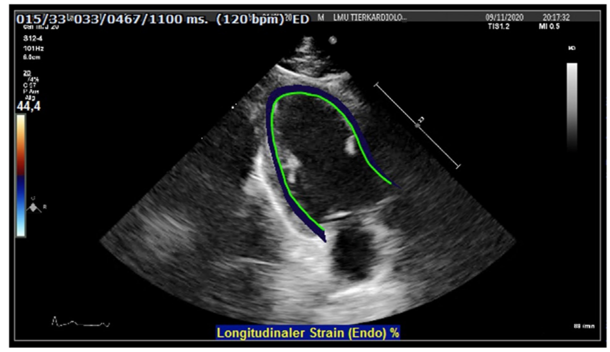

Figure 1 Endocardial tracking of the longitudinal strain in a left apical three-chamber view.

Two-dimensional longitudinal strain measurements were obtained from a left apical two-chamber view, left apical three-chamber view, and a left apical four-chamber view using images with a frame rate of at least 100 Hz. Strain analysis based on two-dimensional STE was performed offline by one investigator using specialized software[b]. For that purpose, cardiac performance analysis (CPA)[b] was conducted including the analysis of all three views.

Tracking was performed using a three-point method. Following this method, one point was placed at the level of the lateral hinge point of the mitral valve, another one at the level of the septal hinge point of the mitral valve, and a final one at the left ventricular apex. All points were placed on the endocardium. Endocardial tracking of a left apical three-chamber view can be seen in Figure 1.

Electrocardiogram tracings were reviewed, and timepoints of respective R-waves, ventricular end-systole, and ventricular end-diastole were adjusted manually if deemed necessary. Analysis was performed automatically by the software, and a manual adjustment of the endocardial border was done if required to ensure an accurate tracking.

The left ventricular free wall as well as the interventricular septum were divided into three parts by the software: basal, middle, and apical. For each segment, endocardial longitudinal strain values were generated, as visualized in Figure 2, and an average was calculated for each view. Endocardial GLS values were obtained with respect to the whole deformation of the left ventricle.

[a] EPIQ 7, Philips GmbH Market DACH, Hamburg, Germany.

[b] TOMTEC Imaging Systems GmbH, Unterschleissheim, Germany.

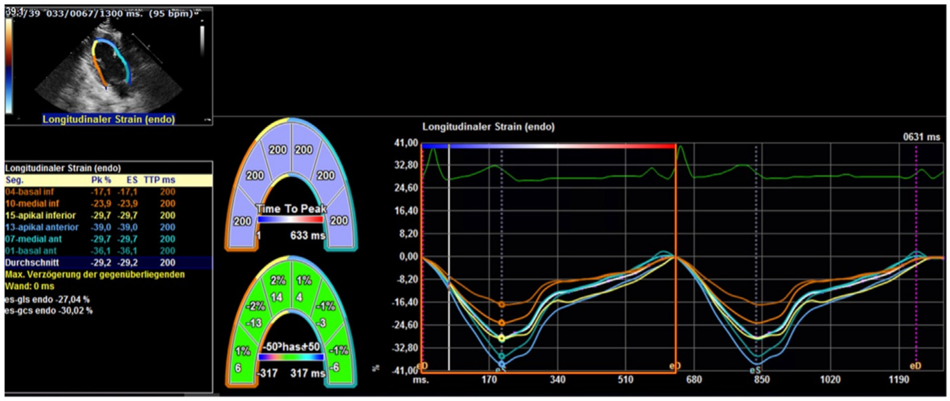

Figure 2 Endocardial longitudinal strain during one cardiac cycle assessed in a left apical two-chamber view. The differently colored lines represent myocardial segments. Segmental values are shown in the left corner.

Statistical analysis

For statistical analysis, specific statistical software[c] has been used. Parameters have been tested for normal distribution using the Shapiro-Wilk test.

The interobserver and intra-observer variability were analyzed using coefficient of variation as well as intraclass correlation coefficient. Ten cases were selected, and endocardial GLS measurements were obtained by the same investigator at three different days blinded to the former results for intra-observer measurement variability. Ten different cases have been analyzed for endocardial GLS values by three different investigators, a board-certified cardiologist, a cardiology resident, and a trained cardiologist. Each investigator was blinded to the others' results. Linear regression models and Bland-Altman plots were evaluated for potential influences of body weight, age, or heart rate.

All observed variables showed weight independence so that regression models for the basis of a non-parametric approach were used. The sample size exceeded 120 individuals so that the Clinical and Laboratory Standards Institute guidelines C28-A2 and C28-A3 for estimating percentiles and their confidence intervals were followed. Values of endocardial longitudinal strain values in different echocardiographic views have been tested for sphericity. Univariate analysis of variance with repeated measures was performed with Huynh and Feldt correction. Post hoc analysis with Bonferroni correction was performed for paired comparisons.

[c] MedCalc, Software Ltd., version 19.5.3, Ostend, Belgium.

Results

The prospectively acquired study population consisted of 132 adult healthy cats with an age ranging between 1.01-19.71 years (5.29 years ± 4.47 years) and a weight between 2.56-7.00 kg (4.38 kg ± 1.05 kg). Fifty-seven cats were male, and 25 were female.

Ten different breeds were included: European shorthair cats (n = 45), British shorthair cats (n = 19), Maine coon cats (n = 19), Norwegian forest cats (n = 16), Bengal cats (n = 13), Siamese cats (n = 5), ragdoll cats (n = 5), mixed-breed cats (n = 4), Birman cats (n = 3), Siberian forest cats (n = 3), a Chartreux cat (n = 1), and an Oriental shorthair cat (n = 1). A total of 19 cats were not included in the study because of insufficient image quality.

The two-dimensional echocardiographic measurements of the study population are shown in Table 1.

Heart rate varied between 119-250 beats per minute (mean: 182 beats per minute ± 27 beats per minute). Endocardial GLS was determined using a constant cardiac cycle length in each cat. Frame rate ranged from 101 Hz to 160 Hz. No significant influence of heart rate on endocardial GLS ($P=0.144$) could be found. Neither a significant influence of age ($P=0.075$) nor a significant dependence on weight ($P=0.102$) was detected. Visual inspection of regression graphs confirmed that none of the factors had an influence on endocardial GLS.

For endocardial GLS upper and lower limits were obtained by analysis of two cardiac cycles and calculated using a 95% percentile. A reference interval of −21.18% to −37.50% (±4.12) was calculated with a 90% percentile of −23.03% to −20.36% and −38.01% to −36.51% for lower and

Table 1 Basic data and standard echocardiographic measurements of the study population.

	N	Minimum	Maximum	Mean	SD
Age (years)	132	1.01	19.72	5.29	4.47
Heart rate (/min)	132	119.00	250.00	181.17	27.15
Weight (kg)	132	2.56	7.00	4.38	1.05
LVIDd (mm)	132	10.00	19.90	15.13	1.85
LVIDs (mm)	132	4.90	14.00	8.95	1.50
LVPWd (mm)	132	3.00	4.99	4.26	0.50
LVPWs (mm)	132	4.20	8.90	6.39	0.91
IVSd (mm)	132	2.80	4.90	4.10	0.52
IVSs (mm)	132	3.30	9.10	5.99	1.00
LA/Ao	132	1.00	1.50	1.32	0.13

Abbreviations: Ao: Aorta; IVSd: interventricular septum in diastole; IVSs: interventricular septum in systole; LA: left atrium; LVIDd: left ventricular internal diameter in diastole; LVIDs: left ventricular internal diameter in systole; LVPWd: left ventricular free wall in diastole; LVPWs: left ventricular free wall in diastole.

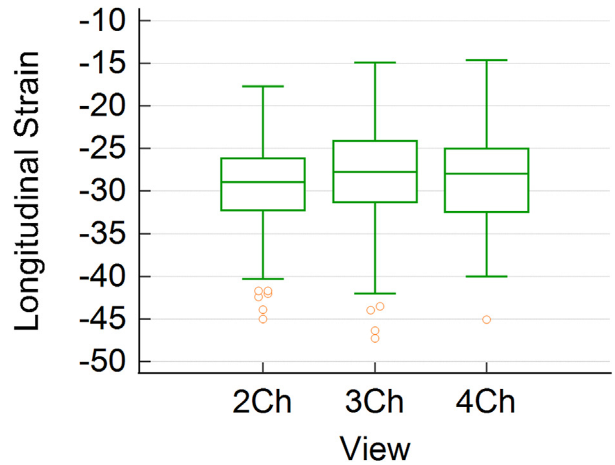

Figure 4 Comparison of the average longitudinal strain of each view, a left apical two-, three-, and four-chamber view. Abbreviations: 2Ch: left apical two-chamber view; 3Ch: left apical three-chamber view; 4Ch: left apical four-chamber view.

upper values, respectively. Data distribution is demonstrated graphically in Figure 3.

There was no significant difference between strain values of the left apical two-, three-, or four-chamber view (P=0.073), as visualized in Figure 4. Tracking of all echocardiographic views was possible for each patient, and a correct tracking was visually verified for each view and each patient. Pairwise comparison between left apical two- and three-chamber views resulted in a P value of 0.126. Similar results were seen comparing a left apical three- and four-chamber views (P=1.000) as well as left apical two- and four-chamber views (P=0.243).

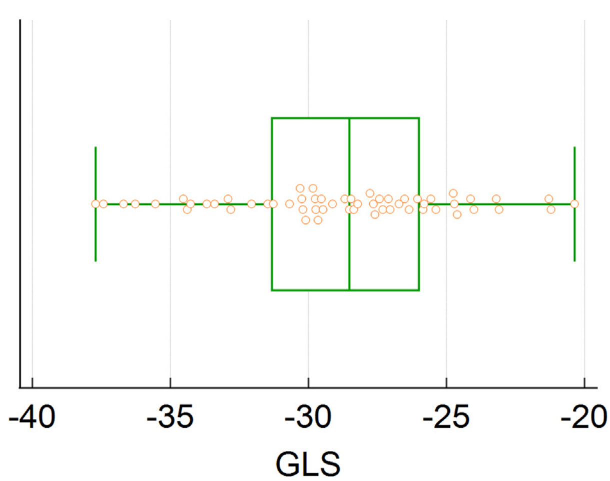

Figure 3 Plot of the endocardial global longitudinal strain representing each measured value. Abbreviations: GLS: global longitudinal strain.

Values of the endocardial analysis of the distinct echocardiographic views ranged from −45.00 to −17.70 (median: −28.95) for the left apical two-chamber view, from −47.30 to −14.90 (median: −27.75) for the left apical three-chamber view, and from −47.10 to −14.60 (median: −27.95) for the left apical four-chamber view.

Measurement variability was excellent with an intra-observer coefficient of variation of 3.50% and an interobserver coefficient of variation of 4.07%. The intraclass correlation coefficient for all three observers was also excellent (0.951), as was the intraclass correlation coefficient for all three measurements of one observer (0.951).

Discussion

The present study establishes reference intervals for endocardial GLS in healthy adult cats using a dedicated, vendor-independent software[b] program for the left ventricle. Following an increasing scientific and clinical interest in feline cardiomyopathies and their etiologies, this study provides the basis for future studies investigating cardiac performance and deformation in various feline cardiomyopathies.

The study shows a very good measurement reliability using two-dimensional STE-derived endocardial GLS, which is important for a potential useful new parameter for the investigation of feline cardiomyopathies. Still, image quality and experience, training, and competence of the investigator are important [35]. Strain analysis and image acquisition are therefore recommended to be done by well-trained specialists to prevent foreshortening among other potential errors [34,35].

Former veterinary studies primarily focused on a left apical four-chamber view to obtain GLS values by two-dimensional STE. However, left ventricular deformation during systole reflects a complex mechanism with motion in multiple planes. To respect the geometrical complex deformation and enhance accuracy and beat-to-beat variation, three different image planes were obtained in this study for endocardial GLS determination, as recommended in human medicine [34].

A gradient with decreasing strain values from the endocardial layer toward the epicardium has been shown [39]. Endocardial strain has currently been recognized as the most sensitive parameter in humans. Moreover, it has been shown that intervendor bias was higher for mid-myocardial GLS than for endocardial GLS [40,41]. Also, in cats, one study showed a good correlation of endocardial strain using two different echocardiographic views in opposite to other layers, which showed significant differences of the layer-specific strain values in both views [29]. Therefore, the present study focused on the endocardial analysis of two-dimensional GLS.

Furthermore, global analysis was preferred over segmental analysis due to previously reported good reliability and repeatability as well as recommendation in human medicine [42,43].

As this parameter might be clinically relevant, good repeatability is of high importance.

As we encourage the thesis of potential regional dysfunction, further analysis of the repeatability of segmental values might be necessary in future studies in cats.

The utilized software[b] provides two modes for strain measurement: Autostrain and CPA. Recently, the automated software for endocardial GLS measurements, Autostrain[b], showed good results in canine patients [44]. However, using Autostrain[b] in our study population at the beginning of the study revealed that tracking of the endocardium was challenging for the software in feline patients with regards to papillary muscles, false tendons, and an overall small size of the heart compared to that of humans and dogs. Therefore, Autostrain[b] was not further evaluated, and the CPA software[b] was used instead. Cardiac performance analysis is a software module allowing more precise cycle adjustment even in high heart rates and imperfect automated electrocardiogram-guided cycle selection. With CPA[b], three or more distinct points need to be selected to guide the tracking.

A good image quality is mandatory for strain analysis because automated tracking could otherwise lead to multiple errors. Extensive manual adjustments should be avoided as it could decrease repeatability of the measurements [35].

The present study did not show a significant influence of heart rate on the endocardial GLS, but it should be taken into consideration that most of the cats had a heart rate above 160 beats per minute. Also, the study population predominantly consisted of young to middle-aged cats, which could lead to an error in the analysis of age as an influencing factor.

Moreover, blood pressure measurement as well as complete blood cell count and serum biochemistry has not been performed in every cat. It might have been necessary to assure their health status with certainty.

Measurement of GLS could also be software dependent, as shown in previous human studies, but attempts have been made by the vendors to ensure that the results are now similar [45]. Despite these efforts, it should be mentioned that the reference range established in this study holds true for the analysis of feline endocardial GLS using CPA software[b]. This software has the advantage that it can be used for vendor-independent analysis and is therefore suitable for echocardiographic core labs analyzing loops from different centers, as recommended for multicenter studies.

This study provides reference intervals for endocardial GLS and therefore sets the ground to be used in further studies to observe and define specific changes in the respective cardiomyopathies.

Conclusion

The present study provides reference values for GLS in a large population of cats. It serves as a basis for investigation of feline cardiomyopathies and early myocardial changes in cats developing various cardiomyopathies.

Limitations

Limitations of our study comprise the following. First, tracking of feline hearts reveals some difficulties given the presence of the small size of the heart. Therefore, papillary muscles and false tendons fall more into account leading to challenging delineation of the endocardium. Second, use of a certain software can lead to the risk of alterations in analysis using different software. Nonetheless, in the past years attempts have been made by the vendors to ensure comparable results. The last potential limitation incorporates the health status of the population, which was assured by medical records and clinical investigation first line. To ascertain the presence of an overall healthy cat, further investigations such as complete blood cell count or serum biochemistry as well as diagnostic imaging might have been favorable. Given no indicators of any illness, stress limitation was preferred with respect to animal welfare.

Conflict of Interest Statement

The authors do not have any conflicts of interest to disclose.

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